บทความวิจัย

ข้าวทับทิมสยาม: การประยุกต์ใช้ในเวชสำอาง

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บทคัดย่อ

ข้าวทับทิมสยามเป็นพันธุ์ข้าวพื้นเมืองของประเทศไทย เมล็ดมีสีแดง นำข้าวที่กะเทาะเปลือกออกแล้วมาบดให้ละเอียด สกัดผงข้าวด้วยเอทานอล จากนั้นวิเคราะห์หาชนิดของสารประกอบในสารสกัดข้าวด้วยเทคนิค GC-MS และ HPLC, ปริมาณสารประกอบฟีนอลิกทั้งหมดด้วยน้ำยา Folin-Ciocalteu, ฤทธิ์ต้านออกซิเดชันด้วยเทคนิค Diphenyl-picryhydrazyl (DPPH) radical scavenging activity assay และ Ferric (Fe^{3+}) reducing antioxidant power (FRAP) assay และคุณสมบัติช่วยสมานแผลด้วยวิธี Scratch Test พบว่าสารสกัดข้าวฯมีสารแอนโธไซยานิน กรดไขมันสายโซ่ยาว และเอสเทอร์ของกรดไขมันสายโซ่ยาวเป็นองค์ประกอบ ปริมาณสารประกอบฟีนอลิกทั้งหมดเท่ากับ 1.5 mg gallic acid equivalent/g rice powder ค่า DPPH radical scavenging activity เท่ากับ 8 mg Trolox equivalent/g rice powder และค่า FRAP activity เท่ากับ 2 mg Fe^{2+} equivalent/g rice powder สารสกัดข้าวที่ความเข้มข้น 25 mg/ml มีฤทธิ์ช่วยสมานแผลเมื่อทดสอบกับเซลล์ L929 ที่ได้รับบาดเจ็บ นำผงข้าวที่ผ่านการสกัดมาอบให้แห้งที่ 50 ^{O}C บรรจุลงในถุงชาขนาดเล็กแบบใช้ครั้งเดียว ของเหลวสำหรับกระจายผงข้าวเตรียมจากสารสกัดข้าว สารอีดีทีเอ กลีเซอรีน โพรพิลีนไกลคอล ไกลเด้นและพอลิไวนิลแอลกอฮอล์ เมื่อต้องการใช้ให้ผสมผงข้าว 1 ถุงกับของเหลวจนได้เพสเนื้อเนียน ทาเพสทั้งหมดลงบนของแข็งค้ำจุนผิวเรียบที่ทาน้ำมันไว้แล้ว เมื่อแห้งลอกฟิล์มที่เกิดขึ้นออก น้ำมันที่ทาลงไปตอนต้นจะถูกดึงออกระหว่างการลอกฟิล์ม สามารถเพิ่มมูลค่าทางเศรษฐกิจของข้าวทับทิมสยามได้โดยผสมลงในเวชสำอาง

คำสำคัญ: ข้าวทับทิมสยาม สารประกอบฟีนอลิกทั้งหมด ฤทธิ์ต้านออกซิเดชัน ฤทธิ์สมานแผล มาส์คพอกหน้าแบบลอกออก

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Tub Tim Siam Rice: Applications in Cosmeceutical Products

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ABSTRACT

Tub Tim Siam rice is an indigenous red rice variety of Thailand. The un-hulled rice was powdered and extracted by using ethanol. The obtained extract was investigated for phytochemicals by using GC-MS and HPLC techniques, total phenolic content (TPC) by using Folin-Ciocalteu reagent, antioxidant activity in regard to DPPH radical scavenging activity and ferric (Fe^{3+}) reducing anti-oxidant power assays, and wound healing property according to the Scratch test. Phenolic compounds, anthocyanins, long chain fatty acids and fatty acid esters were presented in the extract. The TPC value was equal to 1.5 mg gallic acid/g of the rice powder. The DPPH radical scavenging activity and the Fe^{3+} reducing power were of 8 mg trolox equivalent and 2 mg Fe^{2+} equivalent/g, respectively. The extract exhibited wound healing property on injured L929 cells. After extraction, the rice residue was dried at 50^{O}C and packed in single use pouches. Dispersing liquid that contains the extract, EDTA, glycerin, propylene glycol, glydant, and polyvinyl alcohol, was separately prepared and used for dispersing the rice residue in a pouch to obtain smooth paste. Transparent film was formed when left the paste to dry on solid support. Surfaced oil was effectively removed while the film was peeled off. Tub Tim Siam rice might be of economic value when incorporated in cosmeceutical products for improving skin appearance.

Keywords: Tub Tim Siam rice, Total Phenolic Content, anti-oxidant activity, wound healing property, peeling face mask

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Introduction

Pigmented rice is indigenous rice varieties that differ in the bran colors [1]. Tub Tim Siam is red rice variety of Thailand that is colored by anthocyanins. Anti-oxidation and free radical scavenging activity have been reported for such pigments [2]. In human body, free radicals are generated by various metabolic pathways and are stimulated to produce when challenged to hostile conditions [3]. Increased accumulation of free radicals can cause a phenomenon called oxidative stress, which plays a major part in developing chronic and degenerative illness, such as cancer, immune disorders, cardiovascular and neurodegenerative diseases, as well as aging [4-5]. There are defense mechanisms as functioned by superoxide dismutase, catalase, alpha-tocopherol, ascorbic acid, ubiquinone and glutathione to counteract free radicals [6]. Certainly, external antioxidants can be supplied through foods and supplements [7], as well as by using cosmeceutical products [8].

Skin aging is especially important because it represents the sign of aging processes. Physical and psychological stress, alcohol intake, poor nutrition, over-eating, pollutions, and UV radiation may be important factors, which destroy skin structures and cause wrinkles. Delay of the healing process during senility has been a significant impact on wrinkle persistence [9]. In addition, dry skin looks more wrinkled than skin that isn't dry and normal to oily skin is considering firm with fewer wrinkles in compared to dry skin due to its built-in moisturizer. On contrary, excess production of facial oil may induce blemishes and acnes. There are different ways to reduce oiliness on the face. These range from using over-the-counter products to those prescribed by doctors. Purple rice extract has been trialed in 59 women with mild to moderate photodamage for its capability on stimulating production of hyaluronic acid (HA) in skin. Results indicated that HA content in skin was significantly increased after 4 weeks of treatment, suggesting that skin quality were improved [10]. Thus, the rice extract might be useful for treatment of skin aging.

Nowadays, facial masks are innovated by inclusion of active ingredients with immediately perceived efficacy and functionality. In this study, a peeling face mask containing Tub Tim Siam rice extract and the rice residue was developed to help penetration of actives and make immediate radiant of the face after mask removal.

Materials and Methods

Chemicals and reagents

Folin–Ciocalteu's reagent, gallic acid, DPPH (2,2-Diphenyl-1-picrylhydrazyl), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and TPTZ (2,4,6-tripyridyl-s-triazine) were purchased from Sigma-Aldrich (St.Louis, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-dipheniltetrazolium bromide (MTT) reagent was acquired from Bio Basic Inc. (Ontario, Canada). $FeCl_3$ and $FeSO_4$ were obtained from Fine Chemicals (Mumbai, India). Na_2CO_3 was purchased from RFCL Limited (New

Delhi, India). Chemicals and cell culture media were obtained from Thermo Fisher (Waltham, MA, USA). Other used chemicals were of > 99% purity and purchased from Merck (Darmstadt, Germany).

Preparation of Tub Tim Siam rice extract and the rice residue

Dehulled Tub Tim Siam rice bought from Hat-Yai local markets was ground into fine powder. Fifty grams of the powder were soaked in 100 ml of absolute ethanol for 48 h at room temperature with gentle shaking. The supernatant was filtered and evaporated on a water bath at 60^{O}C for 6 h or until viscous extract was obtained. The extract was accurately diluted to 10 ml by absolute ethanol, resulting in 5 g/ml sample stock solution. The rice residue on the filter membrane was collected and dried at 60^{O}C for 2 h in a hot-air oven, cooled in a desiccator, and kept under vacuum at room temperature until use. The percentage of recovery for the rice residue was approximately 99%.

Determination of chemical constituents by GC-MS technique

Chemical constituents in the extract were analyzed by using GC-MS method (Trace GC Ultra/ISQ MS, Thermo Fisher Scientific Inc., US). The capillary column used was 30 m length and 0.25 mm internal diameter with 0.25 μm thickness of AT-WAXMS film. Helium was used as the carrier gas at a flow rate of 1 ml/min. The injection volume was 1μl. The inlet temperature was maintained at 250^{O}C. The oven temperature was initially hold at 80^{O}C for 3 min, ramped to 240^{O}C using a rate of 5^{O}C/min, and hold at 240^{O}C for 15 min. The total running time was 50 min. The MS transfer line and the ion source were maintained at 240^{O}C. Electron ionization was acquired by scanning between 30 and 500 amu. For compound identification, the mass spectra of samples were compared with known spectral database stored in the equipment library.

Determination of chemical constituents by HPLC technique

HPLC technique was additionally used for determining chemical constituents of the extract. The chromatographic system was consisted of a HPLC pump (Jasco PU-1580 Intelligent), a C_{18} column, a 20-μl injector, and a UV/Vis detector (Jasco UV-1575 Intelligent) for measuring the optical density at 254 and 520 nm. The mobile phase was 3% formic acid in methanol and acetonitrile mixed at a 6:4 volume ratio and run at a flow rate of 0.8 ml/min.

Determination of TPC

The TPC was determined by using Folin-Ciocalteu method [11] with some modifications. In brief, 10 μl of sample stock solution was added to a tube containing 70 μl of 10-folds freshly diluted Folin–Ciocalteu reagent and 70 μl of 6% w/v sodium carbonate. After thoroughly mixed and

allowed to stand for 90 min, the OD_{725} was measured against a reagent blank using a microplate reader (Thermo-LUX, Thermo Fisher Scientific Inc., US). Each experiment was performed in triplicate. The calibration curve of gallic acid standard solution in a concentration range of 0.04-0.20 mg/ml in ethanol was prepared. By comparing with the calibration curve, results were calculated and expressed as mg gallic acid equivalent per gram of the rice powder.

Analysis of DPPH radical scavenging activity

The DPPH radical scavenging activity of the extract was determined according to the method previously described [11]. Varying concentrations of the extract was prepared in ethanol. Also, DPPH solution of 0.1 mM was prepared in ethanol. A 40-µl test solution and 160 µl of DPPH solution were thoroughly mixed in a tube. The mixture was kept in the dark at room temperature for 30 min. Then, the OD_{515} was measured using a microplate reader. The calibration curve was prepared using trolox solution in a concentration range of 0.025-0.8 mM in ethanol. Results were calculated by comparing with the calibration curve and expressed as mg trolox equivalent per gram of the rice powder.

Assay of ferric reducing antioxidant power (FRAP)

The method described by Xu *et al.* was used for determining FRAP value of the extract [12]. FRAP reagent consisting of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ with a volume ratio of 10:1:1 was freshly prepared. A 5-µl sample was thoroughly mixed with 150 µl of the reagent and allowed to stand in the dark for 10 min. Then, the OD_{593} was measured using a microplate reader. The solutions of $FeSO_4$ in a concentration range of 0.75–1.75 mM were prepared and used to establish the calibration curve. Results were calculated by comparing with the standard curve and expressed as mg Fe^{2+} equivalent per gram of the rice powder.

Wound healing

The extract was evaluated for wound healing activity as follows:

L929 is mouse fibroblast cell line from the American Type Culture Collection (ATCC). The cells were routinely grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mmol/l L-glutamine and 1% penicillin/streptomycin in a 5% CO_2 incubator at 37^{O}C using standard cell culture techniques. For cytotoxic determination of the rice extract, MTT assay as modified from Graidist et al. [13] was applied. Briefly, the cells of 4×10^5 cells/100 µL were seeded in a well of 96-wells plate and grown overnight. The old medium was changed to the fresh medium containing 25 or 50 mg/ml extract and incubated further for 3 days. After the cultured medium was withdrawn, 50 µl of MTT solution (1 mg/ml in PBS) was added to each well and the wells were incubated for another 4 h in a CO_2 incubator. Excess MTT solution

was discarded and the formed formazan crystal was dissolved by 100 µl/well dimethyl sulfoxide (DMSO). Then, the OD_{570} was measured using a microplate reader. For the control, the extract-free medium was used.

To prepare stress induced cells, the cells of 5×10^6 cells/ml in DMEM were seeded in a well of 6-wells plate and cultured to ~90% confluent. These cells were starved in serum-free DMEM overnight and incubated in DMEM containing 2mM $FeCl_3$ for 30 min. The Scratch test was performed for determining the ability of cell migration after injured by $FeCl_3$, according to the previously described method [14]. In brief, a linear scratch was created on the cell monolayers using a sterile pipette tip. Any cell debris was removed by gently washing with phosphate buffered saline solution (PBS). After that the scratched cells were incubated with a test sample for a desired time period. Photography was carried out by using 4x magnifications of a light microscope on day 1, 2 and 3 of incubation. The distances of each scratched closure from six marked points were recorded and analyzed by using Image J software (National Institute of Health, US). Concerning wound healing property, the percentage of cell migration was calculated by the equation as follows.

$$\% \text{ Migration} = \frac{\text{Average distance of the scratch on day 0 - Average distance of the scratch on day 1, 2 or 3}}{\text{Average distance of the scratch on day 0}} \times 100$$

Development of peeling face mask

Three formulations of facial masks were developed, containing different contents of the rice residue (Table 1). A solid-dispersing solution was prepared as follows. EDTA was dissolved in water to a final concentration of 0.5% w/w. Then, certain amounts of glydant, glycerin, and propylene glycol were added and mixed, and the obtained solution was used for dissolving PVA. Just before use, the rice residue at a desired weight was gradually added in the PVA solution, and the mixture was continually stirred while added. A viscous homogeneous paste was finally obtained, which will be served as a mask product.

Table 1 Compositions of the dispersing solution and mask formulations

	Ingredients of PVA dispersing solution (g)
EDTA	0.5
Glycerin	7.5
Propylene glycol	3.7
Glydant	0.8
PVA	17.5
Water	70
Formulation	Tub Tim Siam rice residue (% w/w)
F1	5
F2	10
F3	15

Oil removing capacity

Water contact angle was applied to determine oil removing capacity of the prepared masks according to Mukherjee et al. with some modifications [15]. Briefly, an oily surface was prepared by spreading homogeneously of 35 mg coconut oil on a 20 cm^2-glass sheet. Thus, each square centimeter of the glass was covered by 1.75 mg oil. Five grams of a formulated mask was applied as a single layer onto the glass to cover an area of 2 cm^2. After allowed to dry for 25 min, continuous and transparent film was formed. The film was detached and water contact angle of the tested surface was measured using Drop Shape Analyzer (DSA100S, KRÜSS GmbH, Germany). The control was the oily surface without mask application.

Statistical analysis

Data were presented as means ± SD with n = 3 and analyzed by using Student's *t*-test on Statistics Package for Social Science (SPSS) software. Statistical differences were considered at $p < 0.05$.

Results and Discussion

Chemical constituents of Tub Tim Siam rice extract

HPLC chromatogram of Tub Tim Siam rice extract is shown in Figure 1. Separation of components in the extract was not satisfied although using the best optimized HPLC conditions. Decreasing or increasing the mobile phase's polarity caused precipitation of the extract. This might

limit optimization of HPLC system. Therefore, identification of any existing compounds by comparing with authentic standards was not performed. Then, the extract was identified and classified into a group of substances in regard to the corresponding absorption chromophore. Phenolic compounds and anthocyanins were present, as a result of light absorption at 254 and 520 nm, respectively [16-17]. Phenolic compounds were certainly determined by the TPC analysis. To precisely determine what compounds are present in the rice extract, GS-MS technique was utilized. The GC chromatogram is shown in Figure 2. Regarding the mass spectra, major ion peaks were detected at m/z 145, 257, 281 285, 309 and 313. By comparing with reference mass spectra as deposited in the instrument, there were long chain fatty acids and ester of the fatty acids, including ethylpalmitate (26.36 min), ethyl octadec-9-enoate (30.27 min), linoleic acid ethyl ester (31.04 min), myristic acid (33.55 min), palmitic acid (37.02 min), 9-octadecanoic acid (42.88 min), and linoleic acid (44.59 min) were detected. In accordance, the extract was likely hydrophobic. Precipitation of the extract seemed to occur when polarity of the mobile phase of HPLC system was increased, for example by increasing MeOH concentration in the mixed solvent (data not shown).

TPC and antioxidant activity

The TPC of the extract was of 1.49 ± 0.23 mg gallic acid equivalent/g of the rice powder. This amount was a little bit low, since the mean TPC reported for red rice varieties has revealed as 4.70 mg gallic acid equivalent/g rice [18]. Nevertheless, great variation in the total phenolic contents for red rice varieties have been documented that may depend on rice genotyping and geography of cultivation areas [14], as well as the polarity of extracting solvents being used [19].

Antioxidant properties of herbal extracts are prior evaluated in order to judge their worth in cosmeceutical perspective. There are methods to be carried out for investigating antioxidant effect of compounds. However, the determinations of DPPH radical scavenging activity and FRAP activity were chosen in this study. The acquired extract exhibited DPPH radical scavenging activity of 8.0 ± 1.50 mg trolox equivalent/g and FRAP activity of 2.03 ± 0.46 mg Fe^{2+} equivalent/g. It is true that ethanol is recommended for use as a solvent for cosmeceutical products, although its extractability and selectivity for antioxidant compounds in the rice might be inferior in compared to other organic solvents, such as methanol, chloroform, diethyl ether and hexane [19]. Moreover, there is difficulty in comparing our data regarding antioxidant activity with those reported in publications. This is because a range of units for presenting antioxidant activity has been found due to using different assay methods and reference standards. However, it was noted that the prepared extract showed antioxidant activity. Whether powerful it is will be examined by evaluation of wound healing property on L929 cells that grown under normal and stress-induced conditions.

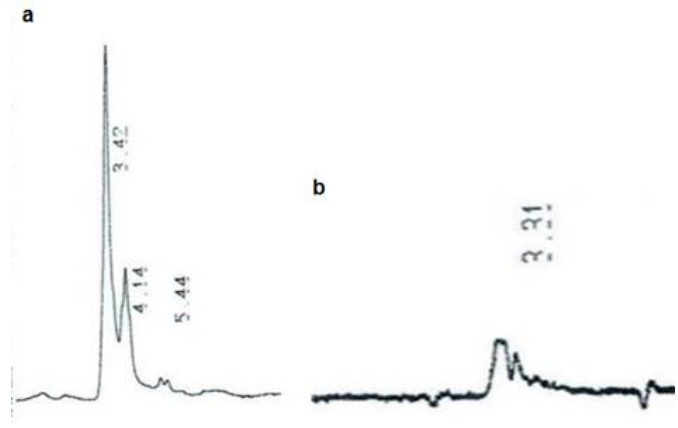

Figure 1 HPLC chromatograms of Tub Tim Siam rice extract using a C18 column, a mobile phase composed of 6:4 volume ratio of 3% HCO_2H in MeOH and CH_3CN run at a flow rate of 0.8 ml/min, a 20-µl injector, a UV-VIS detector monitored at 254 nm (a) and 520 nm (b)

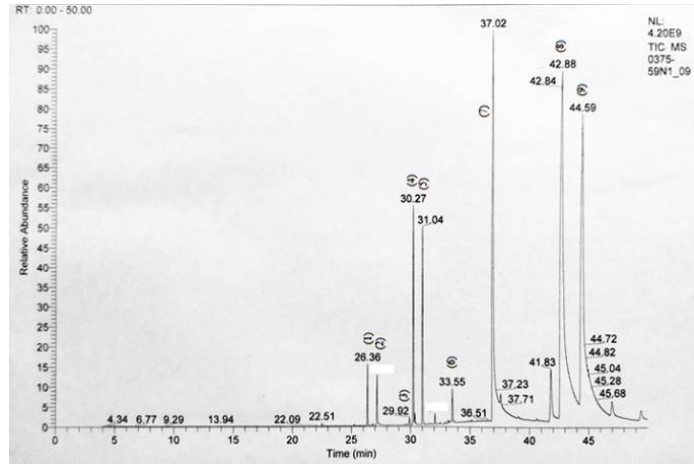

Figure 2 GC chromatogram of Tub Tim Siam rice extract; The chromatographic system was composed of a 30m x 0.25mm capillary column coated with 0.25 µm thickness of AT-WAXMS film, a carrier gas using helium at a flow rate of 1 ml/min, a 1-µl injector with inlet temperature of 250^{O}C, a temperature program hold at 80^{O}C for 3 min, ramped to 240^{O}C at a rate of 5^{O}C/min, and hold at 240^{O}C for 15 min, temperature of MS transfer line and the ion source set up at 240^{O}C. Peaks: ethylpalmitate (26.36 min), ethyloctadec-9-enoate (30.27 min), linoleic acid ethylester (31.04 min), myristic acid (33.55 min), palmitic acid (37.02 min), 9-octadecanoic acid (42.88 min), and linoleic acid (44.59 min),

Cytotoxic evaluation

Live cells can actively metabolize MTT agent to be formazan crystals by using mitochondrial enzyme, whereas death cells are deficient in such ability. The purple color of formazan is optically measured at 570 nm after dissolved by DMSO. The percentage (%) of viable cells following treated with the extract was calculated in compared to the un-treated control. Concentrations of the extract causing cell death of less than 10% were selected for the next investigations regarding wound healing property. In Figure 3, the % viability was insignificantly decreased for the cells incubated with 25-75 mg/ml extract. By using 100 mg/ml extract, great reduction of the cell viability was determined. To maintain minimum cost when the extract is applied commercially and to keep minimum change in osmotic pressure of cell culture medium, only a concentration level of 25 mg/ml will be tested for wound healing.

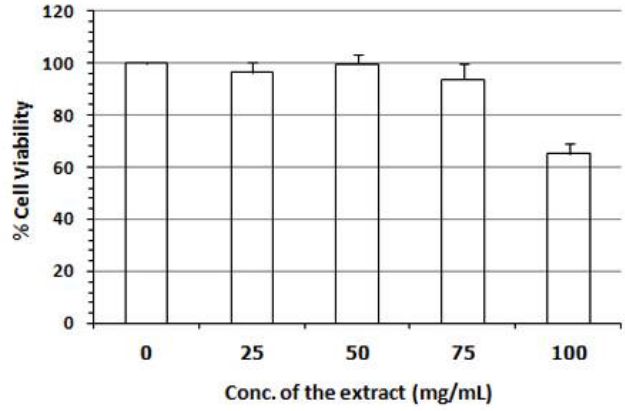

Figure 3 The percentage of cell viability for L929 cells after treated with Tub Tim Siam rice extract of 25, 50, 75, and 100 mg/ml in compared to the un-treated control (0 mg/ml of the extract)

Wound healing property

The ability of cells to migrate perpendicular to the scratched line after treatment with the extract was compared to that of the un-treated controls, and results were expressed as the percentage (%) of cell migration. Iron (Fe) is a crucial element for cell survival and plays a role in driving redox reactions in cells. However, an imbalance of Fe^{2+}/Fe^{3+} concentrations will cause cytotoxic, due to increased free radicals production and oxidative stress. In fact, the human body possesses elegant and elaborate control mechanisms to maintain iron homeostasis [20]. Since the extract exhibited FRAP activity around 2 mg Fe^{2+} equivalent/g, there is an interest to determine wound healing effect of the extract on L929 cells using the Scratch test method. To be certain, the cells were grown normally or made oxidative stress by incubation with 2mM $FeCl_3$ for 30 min before treatment.

Indeed, the number of Fe^{3+}-induced cells was a little bit lower than that of the un-treated controls, suggesting stress inducing effect of Fe^{3+} as mentioned above. However, in regard to % migration, wound healing property of any test samples can be estimated.

Under normal growth conditions, the % migrations of the un-treated cells were of 13.41 ± 0.80%, 29.85 ± 1.61% and 32.79 ± 4.44% after 1, 2 and 3 days of observation, respectively (Figure 4). After treatment with 25 mg/ml extract, increased migration was demonstrated although insignificantly, corresponding to 14.68 ± 3.51%, 32.66 ± 3.46% and 35.64 ± 4.33% on day 1, 2 and 3, respectively. However, the migration of Fe^{3+}-induced cells was impaired if left untreated, as calculated to be of 16.13 ± 5.68%, 18.74 ± 5.00% and 20.53 ± 7.08% for 1, 2 and 3 days of investigation, respectively (Figure 5). Interestingly, the migration of these damaged cells was significantly enhanced by 6.36 ± 4.46%, 18.04 ± 2.37% and 21.71 ± 1.52% after incubation with 25 mg/ml extract for 1, 2 and 3 days, respectively. Thus, the extract was confirmed to have FRAP activity that benefits for healing wounds by decreasing Fe^{3+} induced cytotoxicity.

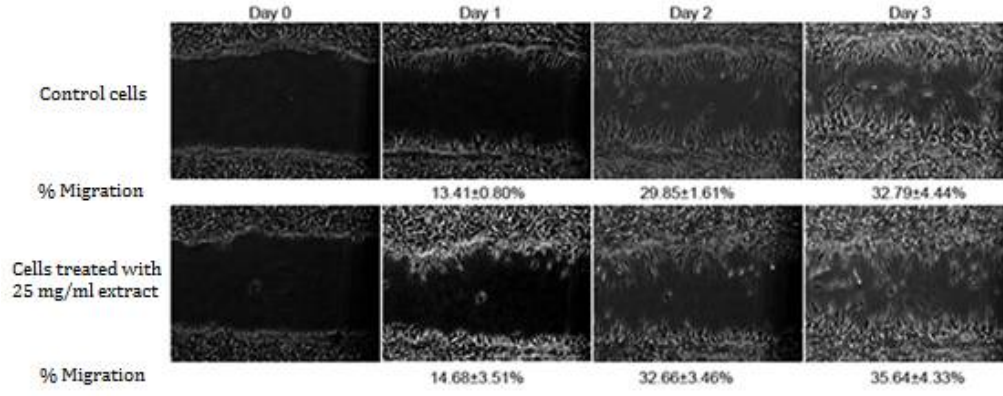

Figure 4 The percentage of migration for L929 cells cultured under normal condition on day 0, 1, 2, and 3 after treatment with 25 mg/ml Tub Tim Siam rice extracts (4X magnifications)

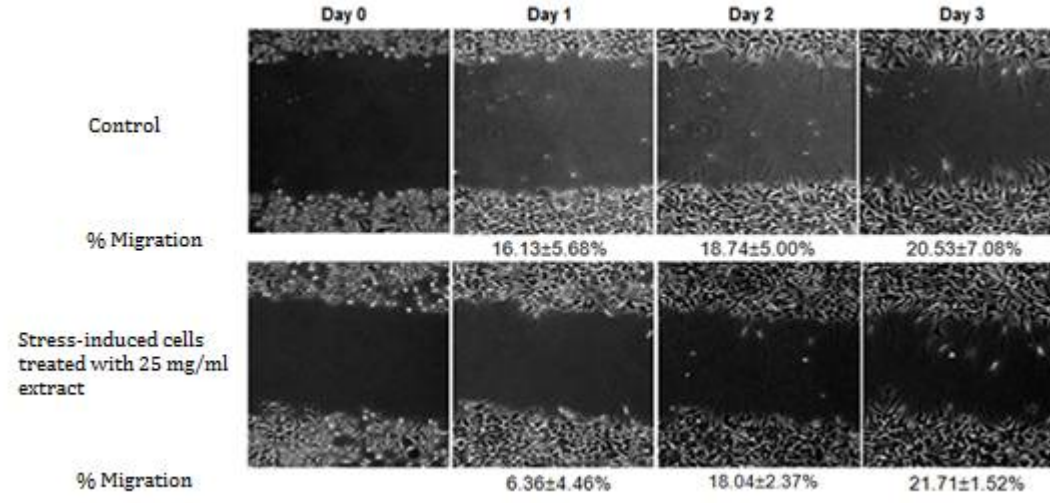

Figure 5 The percentage migration of L929 cells grown under Fe^{3+}- induced condition on day 0, 1, 2, and 3 after treated by 25 mg/ml Tub Tim Siam rice extract (4X magnifications)

Mask formulation and its effectiveness in oil removal

An instant peel-off mask containing Tub Tim Siam rice residue was successfully formulated. Because the rice residue was kept dry in single use pouches and will be dispersed with the providing liquid just before use, this would be advantageous in terms of product stability and microbial contamination. PVA was selected to add in the products because it is the king of film forming agents for peeling face masks [21]. Moreover, it is safe even for irritated skin and can be used in surgical operations [22]. PVA is slowly dissolved in water, but its aqueous solution is stable and transparent. Results showed that the speed of transformation from gel to transparent film could be tailored by changing the amounts of hydrophilic solvents, such as propylene glycol, glycerin, etc. In general, the amount of PVA in mask formulations is between 2.5% and 17.5% w/w [23]. In this work, the concentrations of PVA the finished products were calculated to be of 16.6, 15.8, and 14.9 % w/w, for F1, F2, and F3, respectively. Among these formulations, the time duration required to form film of PVA was not different. This took around 25 $\pm$ 2 min. In accord with the tightening effect of PVA film, actives that presented in the rice residue were assisted to penetrate the skin while the film was being formed. In Figure 6, significant decreasing of water contact angle was observed after the F1 film was peeled from the tested surface. Changes of water contact angle were not apparent while increasing the solid content, as observed for F2 formula. In contrary, increased contact angle was found for F3 mask. Thus, F1 formula revealed sufficient potential in removing surfaced-oil, in compared to the previous study [24] and it might be useful to whom having oily facial skin. Notably, the film of F2 and F3 formulas was characterized to show bumpier surfaces, in compared to that of F1 (data not shown). Good contact between the film that gradually formed and

surfaces underneath was a supposing factor to control oil removing capacity. Nevertheless, mask formulas containing higher rice residue contents may be excellent if nourishing effect is desirable, because actives' concentration in the rice residue would be proportional to its amounts added. By a survey, facial mask formulations in markets today are diverse in terms of actives, major components, indications, application methods, etc., while no certain one has been recommended for use to reduce oily skin. Thus, comparing oil removing capacity between one of these products and that of developed in this study might be not suitable. Even spreading the prepared dispersing liquid on the oily glass, it was found that film of PVA could not be formed (results not shown), suggesting that solid phase like the rice residue was required for the film formation.

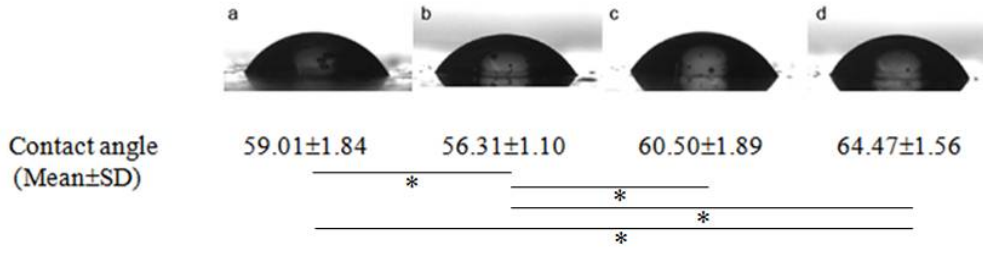

Figure 6 The representatives of water drops captured by a camera of Drop Shape Analyzer: a, the oily surface without any treatment; b-d, the oily surfaces treated with the masks of F1, F2, and F3, respectively. The values of contact angle corresponded to a-d above were indicated as mean $\pm$ SD (n = 3); *, significantly different ($p < 0.05$)

Conclusion

The ethanol extract of un-hulled Tub Tim Siam rice contained substances that exhibited antioxidant and wound healing properties, such as anthocyanins, long chain fatty acids and ester derivatives, as well as phenolic compounds. After extraction, the rice residue might be used for preparing instant mask products. Such development could be a model for inclusion of other actives that might be non-stable or easily contaminated by microorganisms to gain more attractive masks. Nevertheless, Tub Tim Siam rice would be worth for aged and oily skin.

Conflict of interests

The authors declare no conflict of interests.

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